

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of: ) Group Art Unit: 1635  
BECKER *et al.* )  
Serial No. 09/523,237 ) Examiner: Shibuya, M.  
Filed: March 10, 2000 )  
For: KITS FOR AMPLIFYING TARGET )  
NUCLEIC ACID SEQUENCES USING )  
MODIFIED OLIGONUCLEOTIDES )

**DECLARATION UNDER 37 C.F.R. § 1.131**

Box Non-Fee Amendment  
Commissioner for Patents  
Washington, D.C. 20231

Sir:

We, Michael M. Becker, Steven T. Brentano and Mehrdad Majlessi, co-inventors of the above-identified patent application, hereby declare as follows:

1. Prior to August 25, 1995, we conceived of and reduced to practice in the United States modified oligonucleotide primers for use in amplifying target nucleic acid sequences, where the modified oligonucleotide primers contained one or more ribonucleotides having a 2'-O-methyl substitution to the ribofuranosyl moiety. Evidence of this prior conception and reduction to practice can be found in attached Exhibit A, which comprises a set of Steven Brentano's laboratory notebook pages setting forth a study which was conducted to test the efficacy of primers containing 2'-O-methyl substitutions in a transcription-mediated amplification procedure. Although the dates on these pages have been redacted, the study set forth therein was completed in the United States prior to August 25, 1995.

2. The amplification study set forth in Exhibit A included primer sets of both T7 and non-T7 oligonucleotide primers. The T7 primers of this study were 50 bases in length,

Considered KAL 04-12-22

## DECLARATION

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possessed the same base sequence (taking into account DNA/RNA equivalents), and differed in their structures only as follows: (i) the "T7ArpHIV4195(-)" primers contained only unmodified deoxyribonucleotides; (ii) the "T7ArpHIV4195(-)m13" primers contained 37 unmodified deoxyribonucleotides and 13 2'-O-methyl modified ribonucleotides positioned at the 3' most end of these primers; (iii) the "T7ArpHIV4195(-)m18" primers contained 32 unmodified deoxyribonucleotides and 18 2'-O-methyl modified ribonucleotides positioned at the 3' most end of these primers; (iv) the "T7ArpHIV4195(-)r13" primers contained 37 deoxyribonucleotides and 13 unmodified ribonucleotides positioned at the 3' most end of these primers; and (v) the "T7ArpHIV4195(-)r18" primer contained 32 unmodified deoxyribonucleotides with the 18 unmodified ribonucleotides positioned at the 3' most end of these primers. A single non-T7 primer was used in this study, which is identified as the "HIV4116" non-T7 primer.

3. The primers of this study were all tested under essentially identical amplification conditions and at concentrations of 8, 15 or 30 pmol of the T7 primer and 30 pmol of the non-T7 primer in the presence of  $5 \times 10^3$  copies of an HIV target sequence or in the absence of the HIV target sequence. Following the addition of each primer set to an amplification reaction mixture under amplification conditions and for a period of time sufficient to amplify target sequence present in an amplification reaction mixture, a 1  $\mu$ l aliquot of amplification reaction mixture was removed from each 100  $\mu$ l amplification reaction mixture present in each reaction vessel. The 1  $\mu$ l aliquots of amplification reaction mixture were then added to separate vessels, each containing 100  $\mu$ l of deionized water.

4. Amplified target sequence present in the sample of each reaction vessel (either the remaining 99  $\mu$ l of undiluted amplification reaction mixture or the 101  $\mu$ l of diluted amplification reaction mixture) was then determined using a homogenous format described as the Hybridization Protection Assay (HPA) in the instant application, (see specification at page 5, lines 10-18), and acridinium ester (AE)-labeled probes specific for a target sequence present in the

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amplified HIV target sequence. Each sample received 0.1 pmol of AE-labeled probe and 4 pmol of identical cold probe, creating a competition assay as described in the instant application (*see* specification at page 10, lines 19-24). Signal from each sample was measured in relative light units (RLUs) using a luminometer.

5. The results of this study are recorded on page 67 of Exhibit A and are separated into various groupings based on the concentration and structure of the T7 primer tested. Those groups based on the concentration of target sequence present in the amplification mixture are identified as follows: (i) “-” represents the absence of target sequence in the amplification reaction mixture; (ii) “500 copies full format” represents the presence of  $5 \times 10^5$  copies of the HIV target sequence in the amplification reaction mixture prior to amplification and without any subsequent dilution; and (iii) “500 copies 1  $\mu$ l” represents the presence of  $5 \times 10^5$  copies of the HIV target sequence in the amplification reaction mixture prior to amplification, with 1  $\mu$ l of the amplification reaction mixture being diluted with 100  $\mu$ l of deionized water subsequent to amplification and prior to detection. And designations for the T7 primer structures are presented as follows: (i) fully deoxyribonucleotide T7 primers are designated as “N-8”, “N-15” and “N-30”; (ii) T7 primers having 13 3' end 2'-O-methyl modified ribonucleotides are designated as “m13-8”, “m13-15” and “m13-30”; (iii) T7 primers having 18 3' end 2'-O-methyl modified ribonucleotides are designated as “m18-8”, “m18-15” and “m18-30”; (iv) unmodified T7 primers having 13 3' end ribonucleotides, with the remaining bases being deoxyribonucleotides are designated as “r13-8”, “r13-15” and “r13-30”; and (v) unmodified T7 primers having 18 3' end ribonucleotides, with the remaining bases being deoxyribonucleotides are designated as “r18-8”, “r18-15” and “r18-30”. The second number in each case indicates the amount of T7 primer added to the amplification reaction mixture in pmol. All results are presented in terms of (RLUs) detected.

## DECLARATION

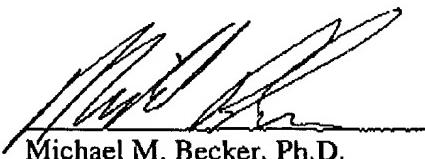
Serial No. 08/893,300  
Atty. Docket No. GP068-02.UT

6. As the results of this study demonstrate, 2'-O-methyl modified primers can be used to successfully amplify target nucleic acid sequences. This is evidenced, for example, by a comparison of results from samples containing no target sequence with samples including either 2'-O-methyl modified primers or unmodified deoxyribonucleotide primers. (It is noted that the excessive RLU value for the "r18-15" sample under the "-" category on page 67 of Exhibit A would suggest that this sample was contaminated with target sequence.) The results for both the 13 and 18 base modified primers in these tests were very similar.

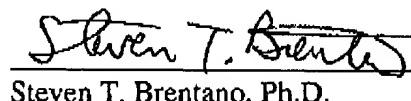
We hereby declare that all statements made herein of our own knowledge are true, and that statements made on information and belief are believed to be true; and further, that these statements are made with the knowledge that willful false statements, and the like so made, are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code, and that such willful false statement may jeopardize the validity of this application and any patent issuing therefrom.

Date: February 4, 2002

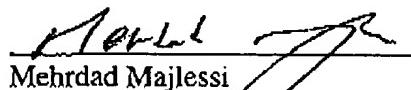
By:

  
Michael M. Becker, Ph.D.Date: 2-4-02

By:

  
Steven T. Brentano, Ph.D.Date: 2/4/02

By:

  
Mehrdad Majlessi

FEB- 6-02 WED 12:17 PM GEN-PROBE

FAX NO. 858 410 8928

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**EXHIBIT A**

TITLE 2'OMe & RNA T7proHIV 4(95E) Primer Test Project No. AMP-T  
 Book No. 8222

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From Page No. 2

Purpose: Test ~~T7~~ <sup>5'</sup> T7proHIV 4(95E) prime w/ either 13 or 18  
 base of 2'OMe or dN4 on 3' end (see 9222:3 & 2830:57).  
 Use lot of target to start. Compare to normal 4(95) primer. Check overall  
 signal & consistency.

Procedure:

- mix w/ all three primers at 8, 15, or 30  $\mu$ l
- use HIV 4(95) as non-T7 at 30  $\mu$ l

	nm	00/ $\mu$ l	nm/ $\mu$ l	nm/ $\mu$ l
<del>T7proHIV 4(95E) n13</del>	nm 3189-33	+7.8	0.712	43.4
T7proHIV 4(95E) n13	nm 3189-41	19.8	0.712	43.4
T7proHIV 4(95E) n18	nm 3189-42	19.75	0.718	48.2
T7proHIV 4(95E) n13	nm 3227-8	6.5	0.26	15.86
T7proHIV 4(95E) n18	nm 3227-6	9.9	0.396	24.16

all 50 long

- non-T7 -

~~T7proHIV 4(95)~~ nm 3097-99 60.0 pmol/ $\mu$ l (30  $\mu$ l)  
 (Abalgene)

- 3(+) & 3(-) angles prime, so make mix  $\approx$  7

- target: use 500 copies target (5 pmol  $\times$  1000  $\times$  3 = 150000 copies/60)

- for 60 of 50  $\mu$ l  $\times$  500 copies =  $3 \times 10^4$  copies in total

- Target =  $2 \times 10^4$  copies/ $\mu$ l  
 $= 2 \times 10^4$ / $\mu$ l

$\rightarrow$  so we 1.5  $\mu$ l + 3 ml H<sub>2</sub>O = 500 copies/50  $\mu$ l

No! 2000/ $\mu$ l  
 so we 500,000 copies!

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Witnessed &amp; Understood by me,

M. P. Bott

Date

Invented by

S. S. Parham

Date

Recorded by

Project No. A.M.P.-T  
Book No. 3222

TITLE cont

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From Page No. 64

- make premix x(15. (105µl) at 2.5µl/loop

287.5 µl AR (950606(X reconstituted))  
5.714 µl F16 (210 µl for 7 loops = 3.49 µl ea)

- ampxmix x7

	x7	T7polx1	T7polx7	T7lx7	premix
W-8	8 µl	56 µl	1.52 µl	12.5 µl	
W-15	15	105	2.05		
W-30	30	210	5.59		
m13-8	8	56	1.29		
m13-15	15	105	2.42		
m13-30	30	210	4.84		
m18-8	8	56	1.16		
m18-15	15	105	2.18		
m18-30	30	210	4.36		
r13-8	8	56	3.53		
r13-15	15	105	6.62		
r13-30	30	210	(3.24		
r18-8	8	56	2.32		
r18-15	15	105	4.35		
r18-30	30	210	8.69		

Lysis  
premix pool

400

- Do ampxmix x7 loops

AR 950606/1X  
MTOJ FR 950302/1X  
MTOJ EOB 9505032X

- 2.5 µl ampxmix/loop (120 loops)

- 50 µl Target on H2O

- 42°C 10 min, 42°C 5 min

- 42°C add 2.5 µl eth. gly (2000 u BT, T1)

- 42°C 90 min

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Witnessed &amp; Understood by me,

Date

Invented by

Date

Recorded by

TITLE cont.

Project No. A99-T  
Book No. 3222

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- HPA 1ul  $\frac{1}{2}$  9.9 ul w/ 4 pmol cold code  
 Uni + 100 ul H<sub>2</sub>O

- probe mix x 160

16 ul 2x hydro  
 $16 \text{ pmol } A6 \text{ probe} = 200 \text{ ul of } 80 \text{ pmol/ul}$   
 $640 \text{ pmol cold code} = 1.5 \text{ ul (at } 425 \text{ pmol/ul)}$

No conc of  
 Uni 80 - really  
 1.5 pmol/ul  
 $\Rightarrow 3.2 \text{ ul of } 5 \text{ pmol/ul}$

- heat 15 min 60°C

- 300 ul out, OH 10 min 60°C

- H<sub>2</sub>O to cool

- read C450 2 sec

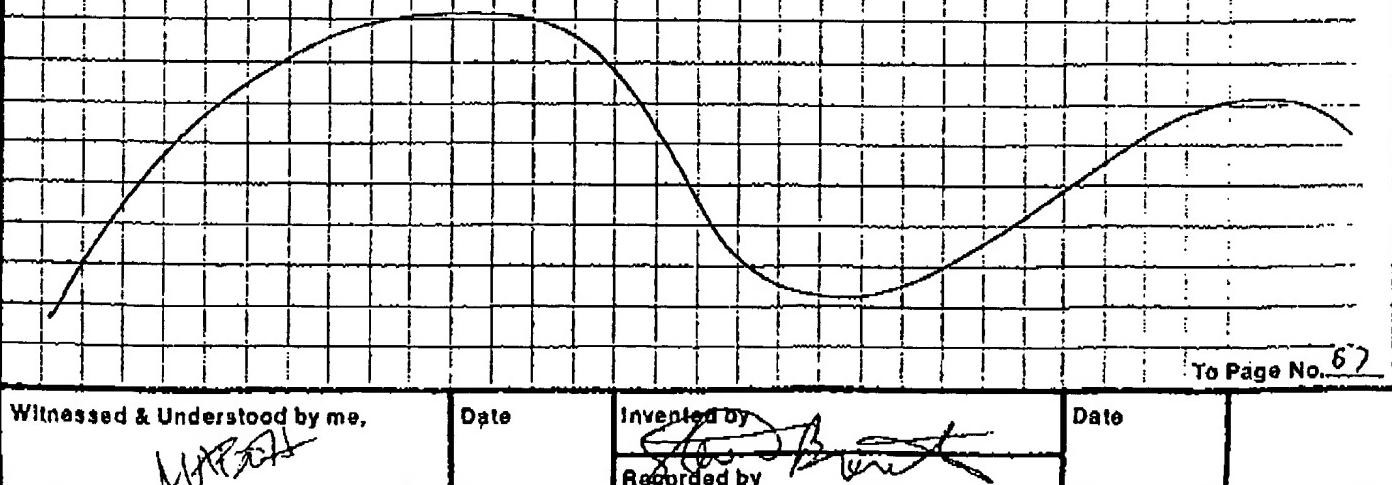
### Results:

- data next page

- all pretty high, ~10,000 RCU  
 use black pipets for next time

- all Normal cRNA & 2' OME primer only worked  
 pretty well & had saturation signal even but only

- try again w/ less target



FEB- 6-02 WED 12:20 PM GEN-PROBE

FAX NO. 858 410 8928

P. 18/18

Project No. A-4-A-T  
Book No. 3222

TITLE Court

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From Page No.		Protocol 1A: BMV DATA 3										Protocol 1B: BMV DATA 2											
		DATE	1	11146	120405	11-30	SAMPLE	21	2704731	11-30	11	2470904	11-30	SAMPLE	1	2464103	11-30						
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TYPE:			23	120405			23	2706120		13	2472276		23	2464103	11-30								
INSTRUMENTS:			24	120405			24	2706120		14	2472276		24	2464103	11-30								
DELAY BETWEEN INSTRUCTIONS:			25	120405			25	2706120		15	2472276		25	2464103	11-30								
DELAY LAST VALUE TO COUNT:			26	120405			26	2706120		16	2472276		26	2464103	11-30								
COUNT TIME:			27	120405			27	2706120		17	2472276		27	2464103	11-30								
BLANK TIME: SUBTRACTION:			28	120405			28	2706120		18	2472276		28	2464103	11-30								
NO. OF COUNTS FOR AVERAGE:			29	120405			29	2706120		19	2472276		29	2464103	11-30								
NO. OF SAMPLE REPETITIONS:			30	120405			30	2706120		20	2472276		30	2464103	11-30								
Protocol 1B: BMV DATA 3												Protocol 1B: BMV DATA 2											
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INSTRUMENTS:			33	120405	11-30	33	2706120	11-30	33	2706120	11-30	33	2464103	11-30									
SYSTEM SERIAL NUMBER:			34	120405	11-30	34	2706120	11-30	34	2706120	11-30	34	2464103	11-30									
CV%: 0.7%			35	120405	11-30	35	2706120	11-30	35	2706120	11-30	35	2464103	11-30									
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TYPE:			38	120405	11-30	38	2706120	11-30	38	2706120	11-30	38	2464103	11-30									
INSTRUMENTS:			39	120405	11-30	39	2706120	11-30	39	2706120	11-30	39	2464103	11-30									
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INSTRUMENTS:			45	120405	11-30	45	2706120	11-30	45	2706120	11-30	45	2464103	11-30									
SYSTEM SERIAL NUMBER:			46	120405	11-30	46	2706120	11-30	46	2706120	11-30	46	2464103	11-30									
CV%: 0.7%			47	120405	11-30	47	2706120	11-30	47	2706120	11-30	47	2464103	11-30									
AVG: 6519			48	120405	11-30	48	2706120	11-30	48	2706120	11-30	48	2464103	11-30									
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SYSTEM SERIAL NUMBER:			52	120405	11-30	52	2706120	11-30	52	2706120	11-30	52	2464103	11-30									
CV%: 0.7%			53	120405	11-30	53	2706120	11-30	53	2706120	11-30	53	2464103	11-30									
AVG: 6519			54	120405	11-30	54	2706120	11-30	54	2706120	11-30	54	2464103	11-30									
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SYSTEM SERIAL NUMBER:			58	120405	11-30	58	2706120	11-30	58	2706120	11-30	58	2464103	11-30									
CV%: 0.7%			59	120405	11-30	59	2706120	11-30	59	2706120	11-30	59	2464103	11-30									
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INSTRUMENTS:			63	120405	11-30	63	2706120	11-30	63	2706120	11-30	63	2464103	11-30									
SYSTEM SERIAL NUMBER:			64	120405	11-30	64	2706120	11-30	64	2706120	11-30	64	2464103	11-30									
CV%: 0.7%			65	120405	11-30	65	2706120	11-30	65	2706120	11-30	65	2464103	11-30									
AVG: 6519			66	120405	11-30	66	2706120	11-30	66	2706120	11-30	66	2464103	11-30									
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INSTRUMENTS:			69	120405	11-30	69	2706120	11-30	69	2706120	11-30	69	2464103	11-30									
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AVG: 6519			72	120405	11-30	72	2706120	11-30	72	2706120	11-30	72	2464103	11-30									
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CV%: 0.7%			77	120405	11-30	77	2706120	11-30	77	2706120	11-30	77	2464103	11-30									
AVG: 6519			78	120405	11-30	78	2706120	11-30	78	2706120	11-30	78	2464103	11-30									
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AVG: 6519			84	120405	11-30	84	2706120	11-30	84	2706120	11-30	84	2464103	11-30									
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SYSTEM SERIAL NUMBER:			88	120405	11-30	88	2706120	11-30	88	2706120	11-30	88	2464103	11-30									
CV%: 0.7%			89	120405	11-30	89	2706120	11-30	89	2706120	11-30	89	2464103	11-30									
AVG: 6519			90	120405	11-30	90	2706120	11-30	90	2706120	11-30	90	2464103	11-30									
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TYPE:			92	120405	11-30	92	2706120	11-30	92	2706120	11-30	92	2464103	11-30									
INSTRUMENTS:			93	120405	11-30	93	2706120	11-30	93	2706120	11-30	93	2464103	11-30									
SYSTEM SERIAL NUMBER:			94	120405	11-30	94	2706120	11-30	94	2706120	11-30	94	2464103	11-30									
CV%: 0.7%			95	120405	11-30	95	2706120	11-30	95	2706120	11-30	95	2464103	11-30									
AVG: 6519			96	120405	11-30	96	2706120	11-30	96	2706120	11-30	96	2464103	11-30									
Protocol 1B: BMV DATA 3												Protocol 1B: BMV DATA 2											
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INSTRUMENTS:			99	120405	11-30	99	2706120	11-30	99	2706120	11-30	99	2464103	11-30									
SYSTEM SERIAL NUMBER:			100	120405	11-30	100	2706120	11-30	100	2706120	11-30	100	2464103	11-30									
CV%: 0.7%			101	120405	11-30	101	2706120	11-30	101	2706120	11-30	101	2464103	11-30									
AVG: 6519			102	120405	11-30	102	2706120	11-30	102	2706120	11-30	102	2464103	11-30									
Protocol 1B: BMV DATA 3												Protocol 1B: BMV DATA 2											
NAME:			103	120405	11-30	SAMPLE	104	2706120	11-30	SAMPLE	105	2706120	11-30	SAMPLE	13	2464103	11-30						
TYPE:			104	120405	11-30	104	2706120	11-30	104	2706120	11-30	104	2464103	11-30									
INSTRUMENTS:			105	120405	11-30	105	2706120	11-30	105	2706120	11-30	105	2464103	11-30									
SYSTEM SERIAL NUMBER:			106	120405																			

To Page No.

**Witnessed & Understood by me,**

Date

Invented by

Date

Recommended by